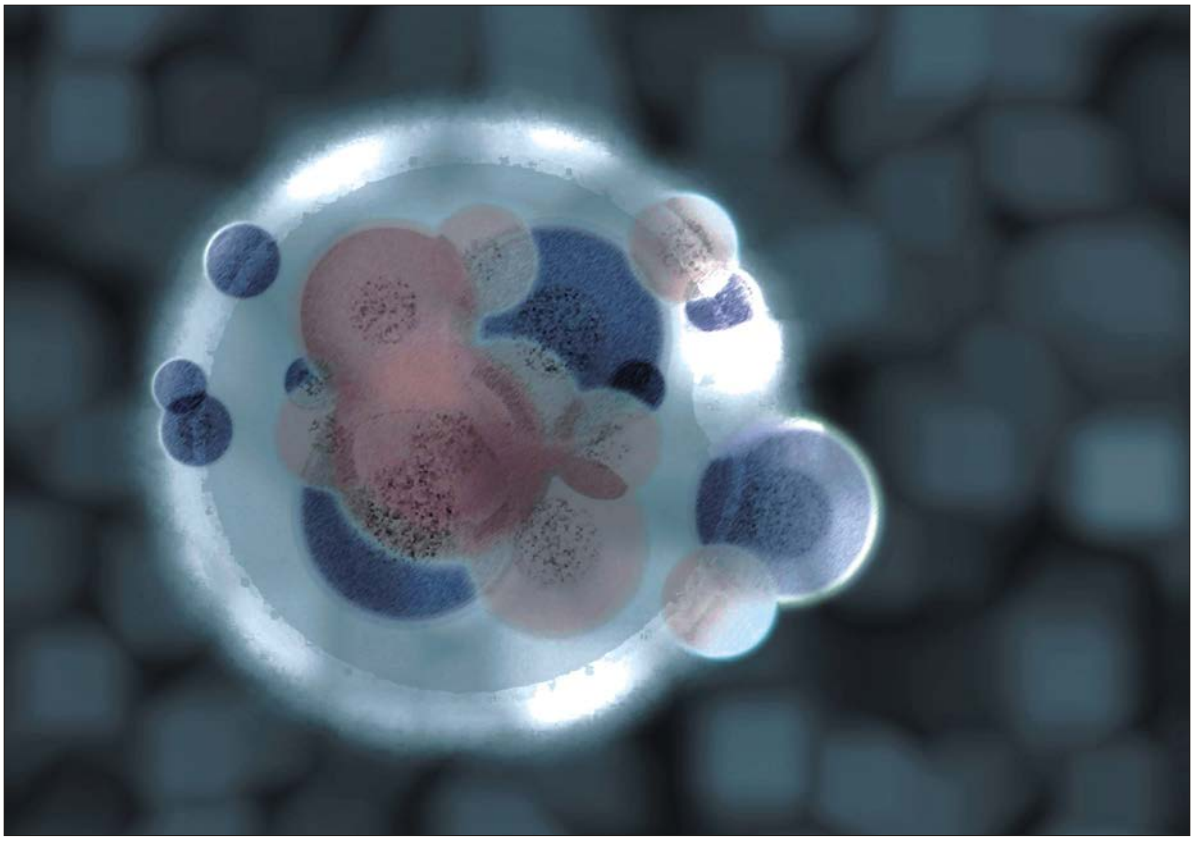

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# Research Article
# Invasive Character of *Prosopis juliflora* Facilitated by its Allelopathy and a Wide Mutualistic Interaction with Soil Microorganisms

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## Abstract

**Background and Objective:** The impact of plant invasions on ecosystems, habitats and native species is severe and often irreversible. The present study attempted to observe the effect of mesquite (*Prosopis juliflora*) on seed germination and plant growth of two native *Acacia* species (*Acacia ehrenbergiana* and *Acacia tortilis*). The effect of mesquite on mutualistic interactions and soil microbial biomass was also investigated. **Materials and Methods:** The effect of *Prosopis juliflora* (*P. juliflora*) extract on seed germination and plant growth of two native legumes was studied in petri dish and pot experiments, respectively. Root nodule bacteria, mycorrhizal fungi and soil microbial biomass were compared between the invasive legume and two native species. **Results:** The results showed that different aqueous extracts of *Prosopis juliflora* significantly affected the germination of native *Acacia* species. Plant growth of these species was also affected by the litter of the invasive plant. A relationship between mutualisms and the invasion process by *Prosopis juliflora* has been recorded. The MPN analysis showed that the density of rhizobia organisms which were able to nodulate *Prosopis juliflora* was greater than for *Acacia* species. Arbuscular mycorrhizae fungi colonization in the roots of native species was affected by *Prosopis juliflora.* **Conclusion:** Results suggested a significant effect of *Prosopis juliflora* on soil microbial biomass. This is the first study reporting the relationship between invasion process of *Prosopis juliflora* and soil microbial community composition in Saudi Arabia.

Key words: Allelopathy, invasive plant, mutualism, soil, *Prosopis juliflora*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Invasive plant species are now considered one of the greatest threats to plant biodiversity. In their new habitat, invasive plant species become predators, competitors, parasites, breeders and disease transmitters for native plants[1]. Invasive plants can destabilize the environment and promote the establishment of other foreign plants[2,3]. Allelopathy is a biological phenomenon by which a plant produces one or more biochemical substance to influence growth, survival and reproduction of other plants. These allelochemicals can positively influence (positive allelopathy) or negative (negative allelopathy) on neighboring plants. Successful establishment of many plants in new environments can be explained by their capacity to produce allelochemical compounds which are toxic to native species[4]. Such compounds are particularly disruptive when they cannot be detoxified by soil microflora. Furthermore, it has also been shown that invasive plants affect soil rhizosphere microflora and alter plants-microbes mutualism[5,6]. Given the apparent role of mutualistic soil microbes in maintaining/determining plant community diversity and structure[7], it is surprising that few studies have explored changes in their interactions with plants within communities in response to invasion[8]. Plant invasions can greatly impact soil microbial community composition and function and consequently, the above-ground structure and composition of native plant communities[9]. Furthermore, plant-microbe mutualistic interactions often facilitate invasions. The symbiotic association with both rhizobia and arbuscular mycorrhizae (AM) can be a major advantage for invading legumes.

Symbiotic relationships between legumes and rhizobia represent an ideal system to study the effects of invasions on below-ground plant-mutualism interaction networks. Rhizobia are capable of establishing symbiosis with legumes by entering their roots and inducing the formation of nitrogen fixing root nodules[10]. Native plants can be the source of symbiotic rhizobia for the invasive species[11]. The capacity of many introduced legumes to nodulate abundantly in new environments could be explained by the presence of a cosmopolitan rhizobial symbionts with a vast host range[12]. An increasing number of studies indicate that nodulation plays an important role in the invasion process[13,14]. Invasive plants also form symbiotic relationships with AM fungi (AMF) which facilitate phosphorus (P) uptake and plant growth. Although AMF can associate with a wide variety of plants from various geographical regions, the success of invasive legumes may depend on their ability to form effective symbiotic relationships with resident AMF populations of native ecosystems. Mycorrhizal colonization improves growth and nutrition of an invasive legume[15]. Actually the distinct advantage of invasive legumes may be attributed to tripartite rhizobia-AMF-plant symbiosis.

*Prosopis juliflora* is a vigorous evergreen tree belonging to the Leguminosae family which was introduced to Saudi Arabia from the Americas. Since it became invasive taking over large swaths of the wooded areas in Tihama plains and elsewhere at the expense of endogenous, well-adapted *Acacia* species[16]. *Prosopis juliflora* is a significant invasive plant in Asia, Australia and also Eastern and Southern Africa, where it impacted plant communities, soil microflora and ecosystem function[17,18]. Several countries attempted to check through the spread of this species eradication[19]. In Ethiopia and Sudan, mechanical methods were used, the trees were uprooted mechanically with machines or by hand. In other countries such as Yemen and Australia, chemical methods (kerosene or diesel oil application followed by burning) were used to kill the plants. In Australia, insects have been used as biological control agents against *P. juliflora.* Unfortunately, these methods don't give the expected result, expensive and mostly ineffective[20]. In order to explain why *P. juliflora* is a better competitor than native tree species, the present study aimed to (i) Evaluate the effect of *P. juliflora* on seed germination and plant growth of two native woody legumes *Acacia tortilis* (*A. tortilis*), *Acacia ehrenbergiana* (*A. ehrenbergiana*) and (ii) Study the effect of *P. juliflora* on mutualistic interactions and soil microbial biomass.

## MATERIALS AND METHODS

**Plant materials and preparation of aqueous extracts:** Litter, leaves and roots of *P. juliflora* were collected from different regions of Jazan region of Saudi Arabia (16.8894°N, 42.5706°E) in January, 2017. Approximately 500 g of each plant material was collected by randomly taking fragments from eight adult *P. juliflora* trees. A subsample of 10 g of plant materiel was soaked in 100 mL of distilled water for 24 h, homogenized and filtered with filter paper (Whatman No. 1). The extract was diluted with distilled water to 1:2 (50%) and 1:4 (25%) in order to obtain 3 applied concentrations: 100, 50 and 25%. These aqueous extracts were stored at -10°C. Part of the leaf litter sample was stored until used in pot experiments.

**Germination bioassay:** The goal of this experiment was to examine the effect of aqueous extract of *P. juliflora* on percent germination and germination speed of two native legumes

(*A. tortilis* and *A. ehrenbergiana*). Germination trials were carried out in sterile petri dishes using 1% agar. Four milliliter of each concentration of aqueous extracts were added to each petri dish. The control was treated with 4 mL of sterile distilled water. Three replicates were used for each of the four treatments. Following seed sowing, germinated seeds were enumerated. During 5 days, germinated seeds were daily counted in order to get two variables: Percent germination (final number of germinated seeds×100/total number of seeds) and germination speed (S) calculated using the index provided by Einhelling *et al.*[21]:

$$S = \frac{N1+N2-N1}{2} + \frac{N3-N2}{3} + \ldots\ldots + \frac{Nn-Nn-1}{n} \times 100$$

where, N1, N2, N3, Nn represent the proportion of germinated seeds in day 1,2,3⋯n after the start of the experiment.

**Pot experiments:** Seedling were grown in pots to assess the effects of *P. juliflora* litter on plant growth of two target legumes. Seeds of each target legume were aseptically transferred into pots filled with autoclaved vermiculite. In each pot, 200 g of freeze-dried litter were crushed and mixed with autoclaved vermiculite. Eight replicates were considered. Control pots (without litter) were included. The pots were placed in a growth chamber at 25°C. Plants were watered once a week with a sterilized nutrient solution. Two months latter the plants were harvested, their shoots and roots were separated, rinsed free of vermiculite, dried at 70°C then weighed.

**Estimation of legume-nodulating rhizobia populations:** The enumeration of legume nodulating rhizobia associated with *P. juliflora* and the target legumes was carried out using the most probable number (MPN) method according to Vincent[22]. Soil was collected from three locations in Jazan (Sabia: 17.20°N, 42.62°E, Wadi Jazan: 16.98°N, 42.63°E and Abu Areesh: 17.02°N, 42.92°E). Soil was passed through a 2 mm sieve and stored at room temperature prior to the experiment. Seeds of the *P. juliflora* and the two target legumes were surface-sterilized and germinated in petri dishes. One seedling was aseptically transplanted into plastic pot filled with autoclaved vermiculite. Inoculation was performed 48 h after transfer with 2 mL of diluted soil suspensions. Four replicates were considered for each tree species. The pots were placed in a growth chamber at 23°C with a 14 h photoperiod and watered daily with sterilized distilled water. Thirty days latter, the presence or absence of nodules was recorded and the MPN was calculated according to Bennett *et al.*[23].

**AMF spore isolation:** Soil samples were collected from three sites (described above). For each site, bulk soil and soil from understory of *P. juliflora* was collected. AMF spores were extracted from 100 g soil. AM spores were isolated by wet-sieving and sucrose centrifugation[24]. Quantification was carried out in petri dishes under a stereoscopic microscope. The spore density was expressed as the total number of spores per 100 g of soil[25].

**Assessment of root colonization by AM fungi:** The symbiosis of AMF of two *Acacia* species (*A. tortilis* and *A. ehrenbergiana*) were investigated after seedlings were raised on soils collected under *P. juliflora* trees. Bulk soil was used as a control. Three sites (described above) were considered. The soil was passed through a 2 mm sieve and a mixture of soils from the three sites was used in this experiment. Three repetitions were considered for each soil type (i.e., bulk soil and soil collected from under *P. juliflora*) and for each tree species. Two months later, plant roots were collected, washed with sterile water, cleared by heating in 10% KOH at 90°C for 1 h, bleached by immersion in 10% $H_2O_2$ for 5 min, acidified in dilute HCl and stained with 0.05% trypan blue in lactophenol[26]. Stained roots were checked for AMF infection by examination under a compound microscope[27]. A minimum of 90 root segments per plant were counted. The intensity of mycorrhization (M) was assessed following the method of Trouvelot *et al.*[28].

**Soil microbial biomass:** Soil microbial biomass carbon (Cmic) of soil sub-samples collected from under *P. juliflora*, *A. tortilis* and *A. ehrenbergiana* trees growing in three sites (described above) was determined by the fumigation extraction method[29] using ninhydrin-N reactive compounds extracted from the soils with KCl after a 10 days fumigation period.

**Statistical analysis:** Statistical analyses were performed with a SAS statistical package. The data were subjected to ANOVA test (t-test ANOVA and one-way ANOVA). Comparisons among means were made using the least significant difference at the 5% level of significance ($p<0.05$).

## RESULTS

**Effect of *P. juliflora* aqueous extracts on germination and plant growth of *A. tortilis* and *A. ehrenbergiana*:** *Prosopis juliflora* shoots aqueous extracts affected significantly the germination of the two *Acacia* species (Table 1). For the control, germination was 69 and 74% for *A. ehrenbergiana*,

Table 1: Effect of different concentrations of aqueous extracts of *Prosopis juliflora* on germination of *Acacia ehrenbergiana* (*A. eh*) and *Acacia tortilis* (*A. to*) seeds

| | | | Litter extract concentration (%) | | | Leaf extract concentration (%) | | | Root extract concentration (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | | Control | 100 | 50 | 25 | 100 | 50 | 25 | 100 | 50 | 25 | F |
| Germination (%) | *A. eh* | 69.4±2.7 | 8.3±4.6 | 8.3±4.9 | 11.0±5.4 | 2.7±2.7 | 16.6±4.7 | 16.6±4.7 | 19.4±2.7 | 30.3±2.6 | 35.6±2.6 | 29.55* |
| | *A. to* | 74.9±4.7 | 22.2±2.7 | 27.7±2.7 | 30.5±5.4 | 22.2±2.7 | 27.7±2.7 | 49.8±8.0 | 49.9±4.7 | 61.1±2.7 | 63.8±2.7 | 32.73* |
| Germination speed (%) | *A. eh* | 19.6±0.7 | 2.3±1.2 | 3.0±1.7 | 3.1±1.53 | 0.6±0.6 | 4.5±1.0 | 6.3±1.2 | 5.2±0.5 | 8.6±0.9 | 10.1±0.5 | 33.05* |
| | *A. to* | 22.4±1.2 | 6.1±0.7 | 7.7±0.9 | 8.0±1.2 | 6.1±0.6 | 7.7±0.6 | 13.6±1.8 | 14.3±1.0 | 15.9±1.3 | 18.7±0.6 | 42.66* |

Data are means±standard error of three replicates, *F: Statistic significant at p<0.05

Table 2: Effect of litter of *Prosopis juliflora* on seedling growth of *Acacia ehrenbergiana* and *Acacia tortilis*

| | *Acacia ehrenbergiana* | | *Acacia tortilis* | |
|---|---|---|---|---|
| Parameters | Control | Treatments | Control | Treatments |
| Shoot length (cm) | 64.3 (±4.7) | 57.5 (±3.3) | 61.0 (±1.60) | 48.5 (±2.60)* |
| Root length (cm) | 22.2 (±1.2) | 21.6 (±2.1) | 25.4 (±2.30) | 23.7 (±2.80) |
| Shoot fresh weight(g) | 17.3 (±1.1) | 11.1±(0.7)* | 6.4 (±0.60) | 4.4 (±0.40) |
| Root fresh weight(g) | 2.0 (±0.3) | 1.9 (±0.2) | 0.5 (±0.10) | 0.3 (±0.05) |
| Shoot dry weight(g) | 6.2±(0.5) | 5.1±(0.1) | 3.2 (±0.60) | 2.5 (±0.20) |
| Root dry weight(g) | 0.8 (0.1) | 0.7 (0.6) | 0.2 (±0.02) | 0.2 (±0.06) |

*Significant difference between mean of growth parameter of treated and control seedling by t-test within species p<0.05

*A. tortilis*, respectively. For extract-treated seeds, germination was reduced with increasing concentrations of the aqueous extracts. Litter and leaves seem to have greater inhibitory effects than roots. In comparison to control, germination of *A. ehrenbergiana* decreased about 61% when treated with 50% litter extract and about 53% when treated with 50% leaf extract. The 50% root extraction reduced the germination of *A. ehrenbergiana* and *A. tortilis* by about 39 and 13%, respectively. Germination speed of *Acacia* seeds was also significantly affected by *P. juliflora* aqueous extracts (Table 1). Germination speed of *A. ehrenbergiana* and *A. tortilis* decreased from 19 and 22% (For the control) to 4.56 and 7.73% (when treated with 50% leaf extract concentration), respectively.

Analysis of plant growth show that growth of *A. ehrenbergiana* and *A. tortilis* seedlings was not significantly affected by the litter of *P. juliflora* (Table 2). The only significant effect of litter of *P. juliflora* was a reduction of shoot length of *A. tortilis* and shoot fresh weight of *A. ehrenbergiana*. After 60 days, shoot length of *A. tortilis* and the shoot fresh weight of *A. ehrenbergiana* were 21 and 33% less than control seedlings, respectively.

**Estimation of legume-nodulating rhizobia populations:** The most probable number (MPN) of rhizobia able to nodulate the roots of *P. juliflora*, *A. ehrenbergiana* and *A. tortilis* seedlings growing in three soils prospected in this study presented in Fig. 1. The populations of indigenous legume nodulating rhizobia varied between sites. For all legumes, MPN was

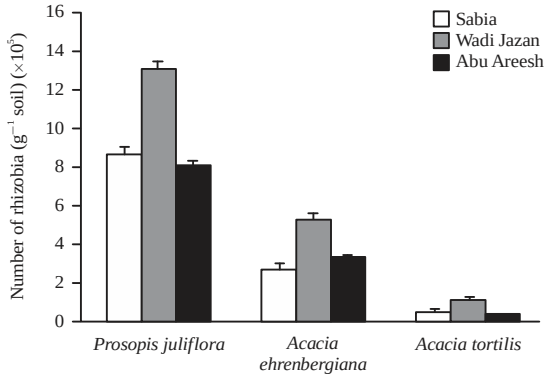


Fig. 1: Most probable number (MPN) of rhizobia able to nodulate *P. juliflora*, *A. ehrenbergiana* and *A. tortilis* in soils from three Jazan sites (Sabia, Wadi Jazan and Abu Areesh)

Error lines correspond to standard deviation (n = 3)

significantly greater in soil 2 (Wadi Jazan) than in soil 1 and soil 3. For all sites, MPN of rhizobia able to nodulate *P. juliflora* was significantly higher than *A. ehrenbergiana* and *A. tortilis.*

**Assessment of root colonization by AM fungi and the number of spores:** The microscopic observations have shown that the roots of all legumes considered in this study were colonized by endomycorrhizal fungi typical hyphae, arbuscules and vesicules were observed in the cortex of the roots of the three legumes, although not necessarily in the same root segment. The intensity of mycorrhization of

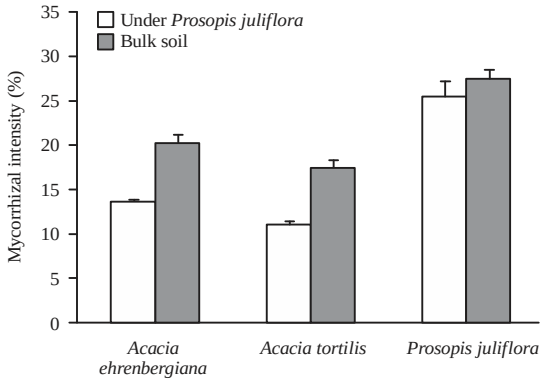


Fig. 2: Mycorrhizal intensity in *P. juliflora*, *A. ehrenbergiana* and *A. tortilis* roots in bulk soil and soil collected from under *P. juliflora*)

Error lines correspond to standard deviation of the means (n=3)

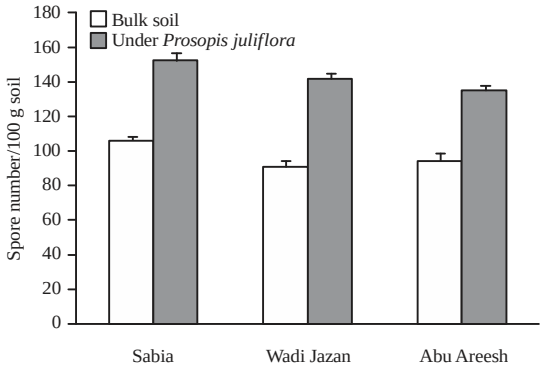


Fig. 3: Spores number of the AM fungi in Jazan soils

Error lines correspond to standard deviation of the means (n=3)

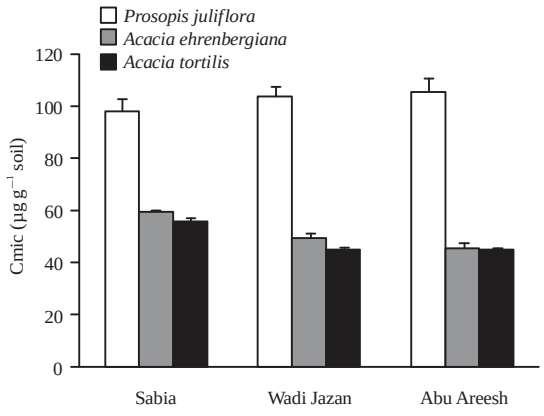


Fig. 4: Rhizosphere soil microbial biomass carbon (Cmic) of *P. juliflora*, *A. ehrenbergiana* and *A. tortilis* collected from three sites in Jazan regions. (Sabia, Wadi Jazan and Abu Areesh)

Error lines correspond to standard deviation (n = 3)

*P. juliflora* was significantly higher than that of native *Acacia* species (Fig. 2). The intensity of mycorrhization of *A. ehrenbergiana* and *A. tortilis* decrease significantly from 20 and 17% (for plant growing on bulk soil) to 13.7 and 11.1%, respectively, for plant growing on soil collected from under *P. juliflora* tree canopies.

Arbuscular mycorrhizal fungal spores were present in all soil samples and the density of spores did not differ among sites (Fig. 3). The majority of spores we collected were small with diameter less than 70 µm, comparable in shape and size to those of *Glomus*. The density of AMF spores varied highly among the treatments from 91 spores per 100 g of soil (bulk soil, Wadi Jazan) to 153 spores per 100 g of soil (in soil collected under *P. juliflora*, Sabia).

**Soil microbiological biomass:** Microbial biomass differed significantly between soils from under *P. juliflora* and native *Acacia* species (Fig. 4). In all sites, Cmic was greater in the rhizosphere of *P. juliflora* than in the rhizosphere of *A. ehrenbergiana* and *A. tortilis.* The highest value of Cmic was recorded in the rhizosphere of *P. juliflora* from Abu Areesh (105.3±5.7). The lowest Cmic was recorded in the rhizosphere of *A. tortilis* from Abu Areesh (44.6±2.5).

## DISCUSSION

In the present study, the allelopathic effect of *P. juliflora* on seed germination, plant growth and microbe mutualisms of two native legumes (*Acacia tortilis, Acacia ehrenbergiana*) was studied.

Seed germination, shoot and root length and shoot and root weight are widely used parameters in allelopathy bioassays[30-32]. In this study, the effect of *P. juliflora* extract on seed germination and plant growth of *A. ehrenbergiana* and *A. tortilis* was studied in petri dish and pot experiments, respectively. The result showed that *P. juliflora* aqueous extracts had deleterious effects on germination of native species seeds. Seed germination and germination speed of *A. ehrenbergiana* and *A. tortilis* were greatly reduced by aqueous extracts of litter and leaves of *P. juliflora.* The germination inhibition increased with extract concentration as was reported by Laosinwattana *et al*[33]. Several previous studies have suggested that the allelopathic effect of *P. juliflora* leaf litter was due to the presence of phenolic compounds[34-36].

Growth of the seedlings of the two *Acacia* species was not significantly affected by the litter of *P. juliflora*. Similar results were reported by Shaik and Mehar[37] for the effect of

*P. juliflora* on germination and plant growth of rice. However, previous study of Alshahrani *et al.*[38] and Mehar[39] reported a higher inhibition of germination and plant growth by *P. juliflora* litter and leaves.

It has been reported that the increased abundance of nitrogen and sulfur caused by the decomposition of litter by soil microbes affects lipid accumulation, acetyl-CoA concentration and acetyl- CoA carboxylase activity[40,41]. Based on these observations, it was suggested that the negative effect of invasive plants on native species was mediated by changes in the microbial communities.

Despite the widely documented and very strong impact of *P. juliflora* on natives species[35,37,39], few reports described the relationship between mutualisms and invasion process of *P. juliflora*. In this study, root nodule bacteria, mycorrhizal fungi and soil microbial biomass were compared between the invasive legume (*P. juliflora*) and two native species.

The most-probable-number (MPN) technique is widely used to enumerate rhizobia based upon the ability of rhizobia to nodulate appropriate host legume plants. This test was based on the assumption that organisms are randomly distributed and that one or more rhizobia are capable of causing nodulation on an appropriate host[42]. In the present study, MPN analysis of whole soil showed that the population density of rhizobia organisms able to nodulate *P. juliflora* was greater than that of the two *Acacia* species. The ability of *P. juliflora* to find compatible rhizobia in introduced regions may be an important factor in the success of the establishment of this legume in many parts of the world. This result was agreed with previous studies which show that many invasive legumes have successfully established symbiotic associations with rhizobia in introduced regions[43-45]. Benata *et al.*[46] described a high diversity of bacteria that nodulate the roots of *P. juliflora* in the Eastern areas of Morocco. This variability in symbiont species and the abundance of nodulation could be considered a major contributing factor to the success of some legume invasive species in colonizing new areas beyond their natural range. Two scenarios are plausible, either *P. juliflora* formed new interactions with native rhizobia or this invasive species was co-introduced with its symbionts. Molecular identification of rhizobia in root nodules of *P. juliflora* should used in order to decide between the two scenarios.

The survey of root mycorrhizal status indicated that the presence of invasive plants decreases significantly the AMF colonization of roots of native species. A higher rate of mycorrhizal colonization was recorded in root of *P. juliflora* compared to roots of native legumes. This suggested that AMF play an important role in the establishment of the invasive legume in new habitats. Given the widespread distribution of AMF and their low-host plant specificity, mycorrhizal associations could favor invasion processes and can be important promoters of plant invasion[12,44,47]. It appears that AMF increase growth and competitiveness of invasive plants[48]. Recently De Souza and Freitas[49] suggested that invasive plants were associated with specific beneficial AMF species, which give them an advantage over native species. Previous reports[50-52] showed that invasive plants negatively affect native plant growth by disrupting their symbiotic associations. Invasive plants produce secondary metabolites that cause changes in soil chemical proprieties that may affect AMF community composition[53,54].

In all studied sites, spore abundance decrease significantly in soil collected from under *P. juliflora* trees as compared to bulk soil. These results were in agreement with previous study of Barto *et al.*[55] and Cantor *et al.*[56] that demonstrated that invasive plants inhibited AMF hyphal growth, spore germination and also altered the AMF community. Plant invasions considerably change the diversity and abundance of soil microbial communities[57-60].

The present study showed that *P. juliflora* affects positively the microbial biomass. The higher microbial biomass was recorded in soil collected from under *P. juliflora*. This could be explained by the modification of the quantity or quality of litter exerted by invasive plants as suggested by Liao and Boutton[61]. Previous studies suggested that invasive plants can change above ground (leaf litter) and below ground (root litter) inputs. Recently, Kuglerova *et al.*[62] found that litter from invasive plants generally decomposed faster than from native species. In this study, *P. juliflora* microbial alterations were mainly driven by leaf litter produced extensively by this legume.

## CONCLUSION

In the present investigation, the allelopathic effect of *P. juliflora* on native woody legumes in Saudi Arabia was documented for the first time. Results showed that *P. juliflora* possessed strong allelopathic potential on *Acacia* species as evidenced especially by the inhibition of seed germination. The inhibition of germination increased with extract concentration. Furthermore, *P. juliflora* affected soil rhizosphere microflora and altered plant-microbe mutualism, especially mycorrhizal associations. This study suggested that relationship between this invasive species and native rhizobia

and AMF facilitate its establishment in new habitats and makes it's a stronger competitor than native plant species.

## SIGNIFICANCE STATEMENT

This study discovers the strong allelopathic effect of *P. juliflora* on native woody legumes. This study will help the researchers to uncover why invasive plants are stronger competitor than native plant species. Thus, find solutions to combat invasive plant species.

## ACKNOWLEDGMENT

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